Oxygen Saturation

A Guide to Laboratory Assessment

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Human life depends on the oxygen transport by hemoglobin. In healthy patients, the majority of molecular oxygen (O_2) is bound to hemoglobin and only a small fraction is dissolved in blood. But in patients with respiratory problems or certain metabolic and genetic disorders, the fraction of oxygenated hemoglobin can fall to dangerously low values. Therefore, laboratory assessment of oxygen saturation (SO_2)—the percentage of hemoglobin saturated with oxygen—provides an important indicator of a patient's cardio-respiratory status and is frequently used in the emergency department, during general and regional anesthesia, and in intensive care settings.

Although the measured parameters are quite different for each, the three major analytical methods for measuring oxygen saturation—arterial blood gas analyzers, pulse oximetry, and CO-oximetry—are frequently used interchangeably by health care workers. Arterial blood gas analyzers calculate estimated oxygen saturation (O_2sat) in a blood sample based on empirical equations using pH and PO_2 values, while pulse oximeters monitor arterial blood oxygen saturation, commonly referred to as S_pO_2 or S_aO_2, noninvasively by passing selected wavelengths of light through an area of the body, such as a finger. Both are measures of oxygen saturation.

The Clinical Laboratory Standards Institute (CLSI) defines O_2sat as an estimated value based on a calculation, whereas S_aO_2 and S_pO_2 refer to the arterial saturation measured spectrophotometrically. (To avoid further confusion, I will use only O_2sat and SO_2 for the remainder of this article.) CO-oximetry, a more complex and reliable method, measures the concentration of hemoglobin derivatives in the blood, the results of which are then used to calculate various parameters such as hemoglobin derivative fractions (e.g., FO_2Hb), total hemoglobin, and oxygen saturation.

In most patients, the results—O_2sat, SO_2, and FO_2Hb—from these three methods are virtually identical. But in cases of dyshemoglobinemia, some methods can yield misleading results, so health care professionals need to understand the differences between these methods and the limitations of each. This article will review the basics of oxygenation of hemoglobin and will provide guidance for laboratorians on how to accurately assess oxygenation saturation values.

An Oxygen Saturation Primer

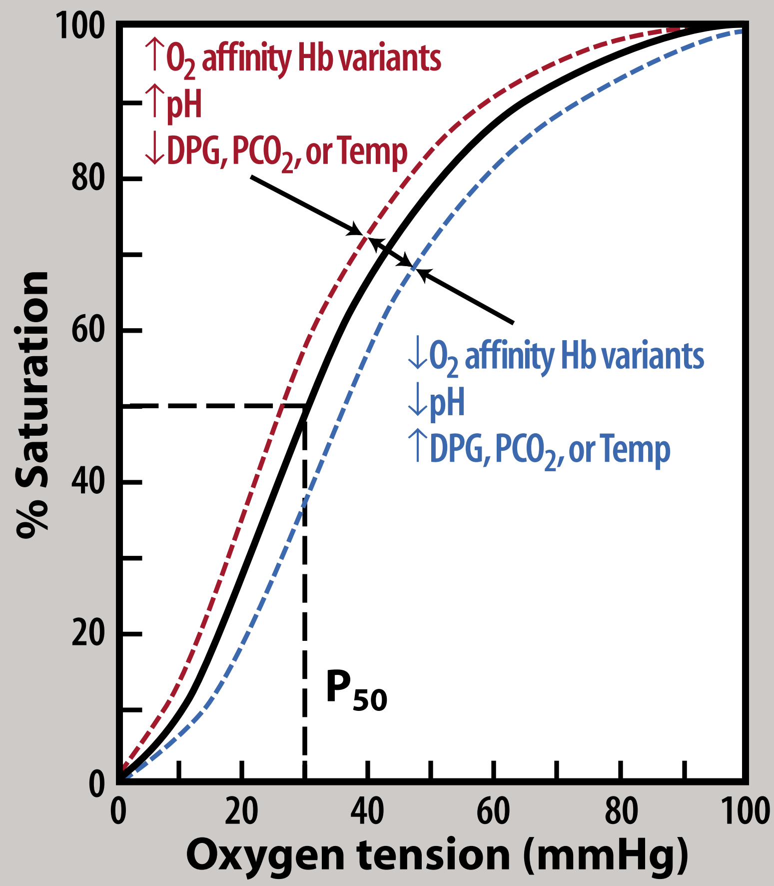
Hemoglobin, the O_2 transport protein in blood, is comprised of four subunits: two α subunits and two non-α subunits, for example ß, γ, or δ. Each subunit contains a porphyrin heme iron (Fe) moiety and seven helices. The heme Fe exists in the Fe(II) or Fe(III) oxidation state, but only the Fe(II) state is capable of binding O_2. Most clinically important dyshemoglobinemia gene mutations, such as the thalessemias, reduce the quantity of α- or ß-chain synthesis in the hemoglobin subunit or lower the solubility of hemoglobin—as occurs with HbS (sickle cell hemoglobin) or HbC (hemoglobin C), for example—but gene mutations rarely alter the O_2 affinity of hemoglobin.

Hemoglobins can be divided into two classes: normal hemoglobin that is capable of binding O_2, and dyshemoglobins—hemoglobin derivatives that are incapable of binding O_2. The normal hemoglobins include oxyhemoglobin (O_2Hb) and deoxyhemoglobin (HHb), while the dyshemoglobins include carboxyhemoglobin (COHb), methemoglobin (MetHb), and sulfhemoglobin (SHb). COHb forms when a person is exposed to CO fumes and CO replaces O_2 in hemoglobin, which can result in death. SHb forms through a reaction of sulfa-containing compounds with the heme moiety; cases of sulfhemoglobinemia are rare and usually result from extensive use of sulfa-containing drugs. MetHb represents the oxidized, deoxy form (Fe(III)-Hb) of hemoglobin to which O_2 cannot bind. The likely etiologies of methemoglobinemia include exposure to highly oxidizing drugs or the presence of genetic hemoglobin variants such as HbM.

To understand how hemoglobin carries and releases oxygen, the oxygen dissociation curve (ODC) serves as an important tool (Figure 1). In the lungs, where partial pressures of O_2 are high, O_2 binds to hemoglobin to form O_2Hb. Erythrocytes carrying O_2Hb then circulate in the blood and release O_2 in response to decreased partial pressures of O_2 in the tissues. The cooperativity of O_2 binding—an allosteric phenomenon whereby binding of oxygen by one hemoglobin subunit enhances the ability of the remaining subunits to bind oxygen—produces the sigmoidal shape of the curve. As O_2 binds to the second and third subunits of hemoglobin, binding increases incrementally so that the four subunits of hemoglobin all become fully saturated at the normal O_2 tension in

Figure 1

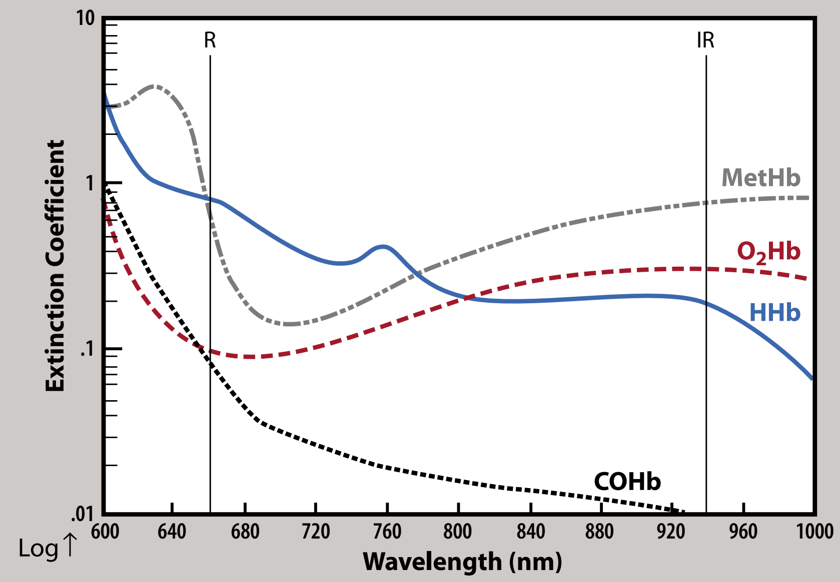
Oxygen Dissociation Curve Of Hemoglobin



The percent saturation of hemoglobin with oxygen at different oxygen tensions is depicted by the sigmoidal curves. The P_{50}, indicated by the dashed lines, is about 27 mm Hg in normal erythrocytes. Modifications of hemoglobin function that increase oxygen affinity shift the curve to the left, whereas those that decrease oxygen affinity shift the curve to the right. Reprinted with permission from *Kelley's Textbook of Internal Medicine, 4th ed.*, 2000, figure 241.2. Lippincott Williams & Wilkins.

Figure 2

Extinction Curves of Purified Hemoglobin Derivatives



The graph shows the light absorption relationship of HHb, O_2Hb, COHb, and MetHb. Vertical lines indicate the red (R) and infrared (IR) monitoring wavelengths used in most pulse oximeters, at 660 nm and 940 nm, respectively. Modified from *Journal of Clinical Monitoring*, 4(4), 1988, p. 292. Pulse Oximetry: Analysis of Theory, Technology, and Practice. M.W. Wukitsch et al. With permission from Kluwer Academic Publishers.

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lung alveoli. The same process works in reverse in the tissues; once fully loaded hemoglobin releases one O_2 molecule, it releases the next more easily.

The ODC also explains what happens to patients when oxygen cannot bind hemoglobin. For example, in the presence of CO or when the heme Fe is oxidized to the Fe(III) state, O_2 cannot bind one of the hemoglobin subunits. These conditions decrease O_2 capacity in addition to inhibiting O_2 transport by blood. In addition, CO and oxidized heme Fe alter hemoglobin's conformation in a way that decreases the O_2 affinity for the remaining heme Fe groups, shifting the ODC to the right. The net biological effect is decreased O_2 delivery to tissues. Temperature, pH, and 2,3-diphosphoglycerate (2,3-DPG) concentration also affect the O_2 affinity of hemoglobin, shifting the ODC as shown in Figure 1.

An important aspect of the ODC is the P50, the partial pressure of oxygen in the blood at which the hemoglobin is 50% saturated. The value for P_{50}—typically about 26.6 mm Hg for a healthy person—is used as an indicator of the affinity of hemoglobin for O_2. In the presence of disease or other conditions that change the hemoglobin's affinity for O_2, the curve shifts to the right or to the left and the P_{50} changes accordingly. An increased P_{50} indicates a rightward shift of the standard curve and means that a higher partial pressure is necessary to maintain 50% oxygen saturation. Increases in temperature and 2,3-DPG concentration, or decreases in pH, can shift the ODC to the right, increasing the P_{50} and indicating decreased O_2 affinity.

Definitions of Oxygenation Status

CLSI has defined the three key terms used to describe oxygenation status: oxygen saturation (SO_2), fractional oxyhemoglobin (FO_2Hb), and estimated oxygen saturation (O_2sat). The Institute recommends the use of the term "oxygen saturation" to indicate the amount of hemoglobin capable of transporting O_2 and "fractional O_2Hb" to represent the fraction of hemoglobin that is oxygenated. The terms "fractional saturation" and "functional saturation" refer to the FO_2Hb and SO_2, respectively.

Oxygen saturation. The following empirical equations are used to determine oxygen saturation, SO_2, from measured hemoglobin concentrations:

Total hemoglobin concentration

$$ctHb = cO_2Hb + cHHb + cMetHb + cCOHb + cSHb$$

Hemoglobin oxygen saturation

$$SO_2 = \frac{cO_2Hb}{cO_2Hb + cHHb}$$

Pulse oximetry and CO-oximetry report SO_2, but with these methods, the values are often referred to as S_aO_2 or S_pO_2. Regardless of the method used, SO_2 is a measure of the fraction of oxygenated hemoglobin in relation to the amount of hemoglobin capable of transporting O_2. The normal range for SO_2, expressed as a percentage, is typically 94%–98%. Blood gas analyzers report an estimated saturation, O_2sat, which is based on measurement of pH, PO_2, and hemoglobin values and utilization of empirical equations.

Fractional oxyhemoglobin. Only instruments with a multi-wavelength spectrophotometer, such as CO-oximeters or some modern blood gas analyzers, are capable of measuring FO_2Hb. The value, as calculated from the formula below, represents the fraction of oxyhemoglobin in relation to the total hemoglobin present, including the non-oxygen-binding hemoglobins.

Fractional oxyhemoglobin

$$FO_2Hb = \frac{cO_2Hb}{ctHb}$$

FO_2Hb is usually expressed as a percentage and typically ranges from 90%–95% in healthy individuals. Reporting FO_2Hb alone is of limited value because the dyshemoglobin fractions also play an important role in the analysis of oxygen carrying capacity. The fraction of any of the hemoglobin derivatives may be calculated in the same manner as that used for oxyhemoglobin.

As demonstrated by the above equations, SO_2 and FO_2Hb are not equivalent terms. In healthy individuals, the values obtained for SO_2 and FO_2Hb are approximately equal, due to the absence of dyshemoglobins. For patients with dyshemoglobinemias, however, FO_2Hb will be considerably lower than SO_2. Although the SO_2 typically remains within the normal range in the presence of elevated COHb or MetHb, the O_2 capacity may be severely decreased, leading to fatal outcomes.

Spectrophotometric Assessment Of Oxygenation Status

Hemoglobin molecules are easily measured by spectrophotometric methods. In addition, the altered molecular structure of the heme moiety in the various hemoglobin derivatives gives rise to unique absorption spectra, making it possible to determine the concentrations of each derivative present in blood. (Figure 2)

Pulse oximetry. Pulse oximeters assess arterial oxygen saturation (SO_2) by measuring light transmission through a well-perfused area of the body such as a finger or an ear lobe. Light sources, typically light-emitting diodes, shine two wavelengths of light through the tissue —visible red (R) light (600 to 750 nm) and infrared (IR) light (850 to 1000 nm). Deoxygenated hemoglobin allows more infrared light to pass through and absorbs more red light than oxygenated hemoglobin, while highly oxygenated hemoglobin allows more red light to pass through and absorbs more infrared light. After calculating the absorption at the two wavelengths, the instrument uses a calibration curve programmed into the device to compute the SO_2.

For example, on most pulse oximeters an absorbance ratio (AR/AIR) of 0.43 corresponds to 100% oxygen saturation, and a ratio of 3.4 corresponds to 0% oxygen saturation. In the absence of a dyshemoglobin, an absorbance ratio of 1.0 corresponds to an oxygen saturation of approximately 85%. As mentioned previously, pulse oximetry SO_2 values can be clinically misleading in the presence of dyshemoglobins. COHb and MetHb greatly decrease the oxygen carrying capacity of blood, but not the oxygen saturation. Consequently, the SO_2 value will be close to normal in these cases. For example, a patient with fractional carboxyhemoglobin (FCOHb) of 20% may have a SO_2 value of 95%, although the FO_2Hb will be near 75%. Therefore, during the initial evaluation of oxygen status in an unconscious, dyspneic, or otherwise impaired patient, health

Table 1

Analytical Methods for Measuring Oxygen Saturation

Device	Specimen	Measurement	Reported Data	Advantages	Disadvantages	Notes
Arterial Blood Gas (ABG) Analyzer	Blood	Partial pressure of oxygen dissolved in whole blood at an electrode	PO_2, SO_2 (O_2sat) Some models available with CO-oximetry capabilities (see below)	Also measures pH and PCO_2	Invasive, O_2sat may be inaccurate in hospitalized patients and if dysHb present	If the ABG and pulse oximetry are discrepant, an abnormal Hb may be present.
Pulse oximeter	Transcutaneous	Absorption at two wavelengths (660 nm and 940 nm) in blood	SO_2	Non-invasive, continuous bedside monitoring	Inaccurate when interfering substances are present: MetHb, certain dyes	When MetHb >25% the pulse oximeter will read saturation of 75%–85%.
CO-oximeter	Blood	Absorption of Hb derivatives using multiple wavelengths of light	SO_2, FO_2Hb, FHHb, FMetHb, FCOHb, FSHb, ctHb	Measures the concentration of Hb species	Invasive, performed in laboratory; not all instruments report SHb, total bili	Most accurate method; even in cases of CO-poisoning and methemoglobinemia. May be inaccurate in cases of HbM.

care professionals should use CO-oximetry to rule out the presence of high fractions of dyshemoglobins. Similarly, the use of pulse oximetry to monitor SO_2 in patients with elevated MetHb is not recommended. When MetHb concentration increases above 35%, the pulse oximetry response reaches a plateau of 84%–86% saturation. At this point, the SO_2 becomes virtually independent of the MetHb concentration; such situations have been noted in several case reports of symptomatic methemoglobinemia in the literature.

The effect on SO_2, if any, of increased fractional sulfhemoglobin (FSHb) or FCOHb is much less pronounced than for elevated fractional methemoglobin (FMetHb). This phenomenon may be explained by examining the spectroscopic signatures of the hemoglobin derivatives. MetHb absorbs light almost equally at the red and infrared wavelengths used by pulse oximeters (Figure 2). Although MetHb absorption at 660 nm is similar to HHb absorption at that wavelength, MetHb's absorption at 940 nm is markedly greater than that of either HHb or O_2Hb. As a result, MetHb will contribute to the perceived absorption of both HHb and O_2Hb. This increases the numerator and the denominator of the ratio between absorption at 660 nm and absorption at 940 nm, causing the ratio to approach unity. As previously mentioned, a ratio of 1.0 corresponds to a saturation of 85% on many pulse oximeter calibration curves, which in the case of a patient with methemoglobinemia could be dangerously misleading.

CO-oximetry. Early CO-oximeters were simplified spectrophotometers that measured light absorbance at four or more different wavelengths chosen to correspond to the specific absorbance characteristics of the hemoglobin derivatives (Figure 2). By the mid-1980s, CO-oximeters were capable of measuring fractions of HHb, O_2Hb, COHb, MetHb, and SHb using six wavelengths. Current models measure absorbance at over 100 wavelengths and are called continuous wave spectrophotometers. The additional wavelengths improve the accuracy of the spectrum, minimize or eliminate the effects of interfering substances, and enable reporting of additional components. More complex CO-oximeters are also available that measure absorbance at 128 wavelengths and can report total hemoglobin concentration (ctHb) and SO_2 in addition to fractional deoxyhemoglobin (FHHb), FO_2Hb, FCOHb, FMetHb, and FSHb.

The assumption that FO_2Hb is equivalent to SO_2 does not hold in cases of cyanosis that are due to increased dyshemoglobin fractions. Consequently, in these cases, labs should not rely solely on SO_2, but should use a CO-oximeter that has been programmed to report FO_22Hb, FCOHb, FMetHb, and FSHb until the presence of dyshemoglobins is eliminated. Furthermore, laboratorians need to be aware that neither SO_2 nor FO_2Hb reflects the total amount of O_2 bound to hemoglobin, as this depends on the PO_2 and the total Hb concentration.

Non-Spectrophotometric Methods of Analysis

Most blood gas analyzers are comprised of a series of electrodes that provide information regarding a patient's acid/base status (pH), respiratory function (PCO_2), and oxygenation status (PO_2). PO_2 measurements are based on changes in electrical current measured at a Clark electrode, whereas pH and PCO_2 measurements are determined from voltage changes at high-impedance electrodes. Newer, point-of-care (POC) blood gas analyzers employ dry slide technology with optical detection.

Some of the parameters measured by blood gas instruments are different than those measured by the spectrophotometric methods. The PO_2 value determined by these instruments refers to dissolved oxygen gas in blood. The concentration of dissolved oxygen (cdO_2) is linearly related to the partial pressure of oxygen in blood according to Henry's law where the solubility constant of O_2 ($\alpha O2$) is 0.03 mL O_2/L/mm Hg:

$$cdO_2 = \alpha O_2 \times PO_2$$

However, the relationship between the estimated SO_2, often referred to as O_2sat, and PO_2 may *not* always be linear. O_2sat is calculated from the pH and PO_2 values and the standard ODC for oxygen saturation. Unfortunately, this approach to calculating O_2sat assumes a normal ODC, which is frequently not the case in hospitalized patients. To circumvent this problem, some labs customize the output of blood gas analyzers so that they do not report O_2sat; however, some POC devices do not offer this option and could potentially mislead some health care professionals. To avoid such errors, the most recent approved guideline from CLSI discourages the use of estimated values.

Best Practice for Oxygen Saturation Measurement

Despite the limitations discussed above, measurement of the oxygen saturation of hemoglobin remains a valuable tool for assessing a patient's respiratory status, and laboratorians can enhance the quality of patient care by being aware of these limitations and educating other health care workers about them. Table 1 summarizes the advantages and disadvantages of the three analytical methods for assessing oxygen saturation. The most important point to note is that the commonly reported parameters from the three methods—O_2sat, SO_2, and FO_2Hb—are quite different and therefore should not be used interchangeably.

In addition, laboratorians and other health care workers need to be aware that estimated O_2sat values should be interpreted with caution as the algorithms used to calculate this value assume that the patient has normal O_2 affinity, normal 2,3-DPG concentration, and no dyshemoglobin or hemoglobinopathy.

Finally, it is important to note that SO_2 has limited value as the sole indicator of oxygen status in cases of suspected dyshemoglobinemia, because the presence of dyshemoglobins affects O_2 capacity and affinity but not saturation. In these cases, information from CO-oximetry is necessary. In addition to the FO_2Hb, CO-oximeters should be programmed to report the various dyshemoglobin fractions in addition to FO_2Hb_1, as FO_2Hb alone has limited utility. CLN

SUGGESTED READINGS

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ACKNOWLEDGMENTS

The author would like to acknowledge the efforts of Drs. Mitch Scott, Chuck Eby, and Rohit Cariappa, who were co-authors on the case conference publication that inspired this article (*Clin Chem* 2005; 51: 434–444).

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Oxygen Saturation *Some Common Abbreviations*

O_2	Molecular oxygen
O_2Hb	Oxyhemoglobin
CO	Carbon dioxide
HHb	Deoxyhemoglobin
SO_2	Oxygen saturation
COHb	Carboxyhemoglobin
O_2Sat	Estimated oxygen saturation
MetHb	Methemoglobin
S_aO_2 or S_pO_2	Arterial oxygen saturation, as reported by pulse oximeters
SHb	Sulfhemoglobin
HbS	Sickle cell hemoglobin
FO_2Hb	Fractional oxyhemoglogin
HbC	Hemoglobin C
cO_2Hb	Oxyhemoglobin concentration
HbF	Hemoglobin F
ctHb	Total hemoglobin concentration
FCOHb	Fractional carboxyhemoglobin